



Workflow Overview

Daily Procedure for SONY SH800S Sorters

Flow Cytometry
Core Facility

Ver. 3
2020-10-19

Initial
Instrument
Setup



Auto
Calibration



Sample
Line
Cleaning



Shutdown

1. Initial Instrument Setup

Perform Initial Instrument setup if you are the first person of the day and:

- It is before 10 AM during weekdays.
- It is a weekend or holiday.

- 1 Open air valve. Turn blue lever on left side of the hood.

CLOSED



OPEN



- 2 Turn on the hood by pressing the following buttons:

AMS Control: Low
Fan + Light + Outlet



3 Clean sort chamber with 70% ethanol and kim wipes.

- Wipe the inside of collection chamber, including the door in front of deflection plates.
- Verify deflection plates are clean. If they are dirty, remove and clean with 70% EtOH.
- Remove any sort collection apparatuses from the sort chamber. This must be done prior to any QC.

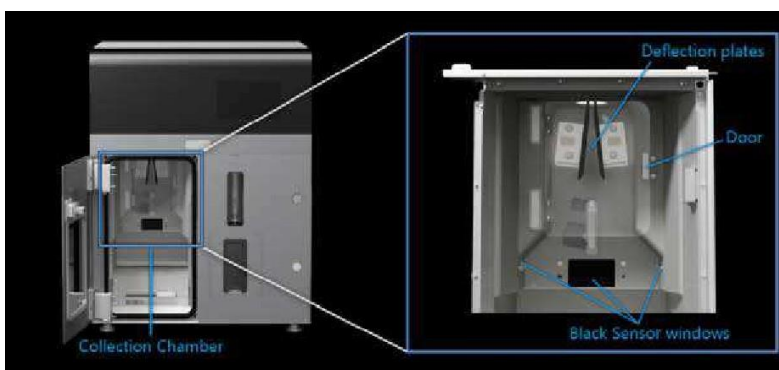


Image taken from Troubleshooting section – Cell Sorter Software

4 Check the fluidics.

- Check if there is sufficient sheath fluid in the sheath tank (a): **Sheath=1xPBS.**
To prepare, combine 200 mL of 10XPBS with 1800 mL of dH₂O or replace with a full autoclaved tank.
- Check if the waste tank is empty (b).
- Check that the DI water bottle is full by opening the door on the left side of the instrument. If needed, fill it with DI water from PureLab system.

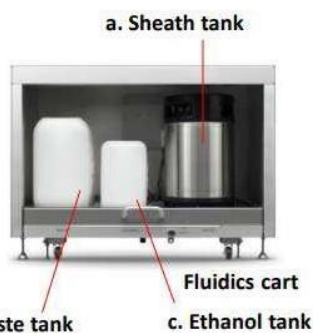
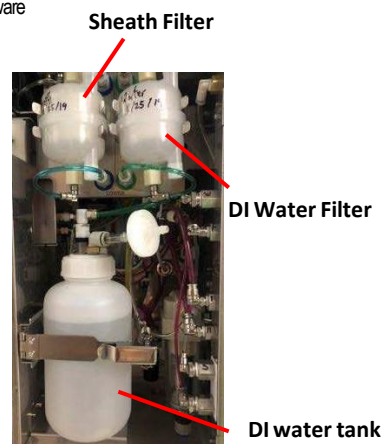


Image taken from SONY® Cell Sorter Operator's Guide



5 Restart computer and Login to Windows

Username:
Password:

6 Turn ON SONY SH800 sorter.

Once computer is fully on - press button in front of the SH800.

Note: The front door can NOT be opened unless the instrument is ON and pressurized.



Power button

Image taken from SONY® Cell Sorter Operator's Guide

7 Launch Cell Sorter Software.

Login name: YourName
Password: unique to user

8 Registering a Chip.

- Check to see if there is a chip available that has been used < 24 hours before with the nozzle size you need. If not, please use new chip.
- Scan the QR code on the sorting chip package in front of the built-in camera on the SONY computer.
- Verify if the info displayed on the screen matches the QR code scanned. Click Next
- If it's the first time scanning it, write down date and time on the cover of the chip and reserve. Do NOT write over QR code.

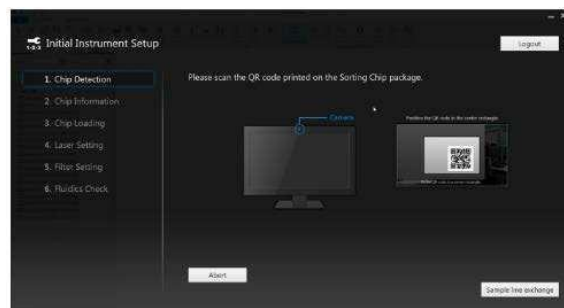
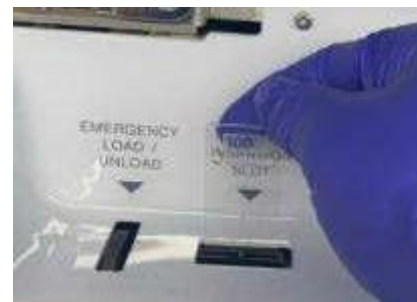


Image taken from

<https://www.sonybiotechnology.com/us/instruments/sh800s-cell-sorter/software/>

9 Loading a Chip

- ✚ Lift the front sash of the BSC.
- ✚ Push to open the top front panel of the SONY.
- ✚ If the ejected chip is going to be reused, reinsert it by applying a small amount of pressure on top of it. To use a new chip, lift the old chip from the insertion slot and insert the chip that was just scanned.
- ✚ When inserting any chip, load with the "# µm" label facing towards you, as shown in the picture. The chip will be loaded automatically. Do not exert excessive force when inserting the chip.
- ✚ Dispose any chip more than 24 hours old as biohazardous waste. For chips that were scanned less than 24 hours ago, place back in it's original sleeve and place in the hood for potential reuse.
- ✚ Click Next.



10 Laser Selection.

Place a check mark on all the lasers. Always set all lasers on as the default setting.

11 Check the Optical Filter Pattern.

- ✚ Select "With 405nm" as this is the default optical configuration.
- ✚ Compare both physical and displayed optical filter pattern. Ensure they match. Click Next.

12 Fluidics Check.

Check if the sample line is dripping. If not, there might be a clog. Follow on screen prompts.

This is an automated procedure, but at this stage (step 6 of initial setup) the sheath filter debubble needs to be done.

- ✚ Select "Sheath Filter Debubble" option.
- ✚ Follow on-screen instructions.
- ✚ When prompted to release trapped air from the filter, tap the filter itself gently and then open the bleed valve to release trapped air. Close the valve and clean any surfaces where PBS might have spilled.
- ✚ Click "Next" if droplet shape is stable for the software to continue the fluidics check.



2. Autocalibration

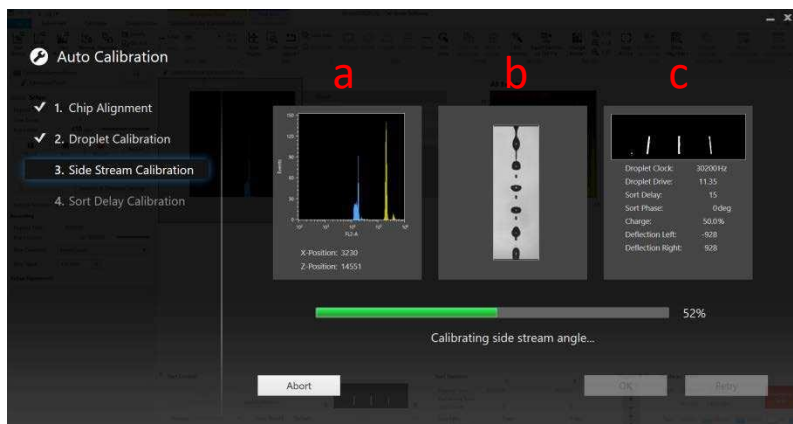
1 Prepare Calibration beads

- ✚ The Automatic setup beads (Part Number: LE-B3001) are located in the FCCF refrigerator.
- ✚ Vortex the vial in use.
- ✚ Put 10 drops of resuspended beads into a 5mL tube. No dilution necessary.
- ✚ Place the beads to tube into the proper adaptor inside the sample loading chamber.
- ✚ Select "OK" to proceed with calibration.

Note: Make sure there is no collection holder in the collection stage.

2 Chip Alignment

- In these steps, the sorter is placing the chip in an optimal position in front of the optical lens. This will ensure that the QC beads are at their brightest with the lowest CV, which will help to obtain the best signal for experimental samples.
- This alignment is completed using beads with a **low** and a **high** fluorescence intensity (a).



3 Droplet and Side Stream Calibration.

- The optimal Droplet Clock frequency and Droplet Drive amplitude are calculated to form stable droplets with an optimized breakoff point that produces stable side streams (b).
- The angle of the side streams for all collection tubes is defined at this step.
Note: Due to side stream validation, alignment only needs to be validated for plate sorting.

4 Sort Delay Calibration.

The optimal drop-charge delay is calculated to ensure the correct droplets are sorted.

5 Automatic Calibration Finishes.

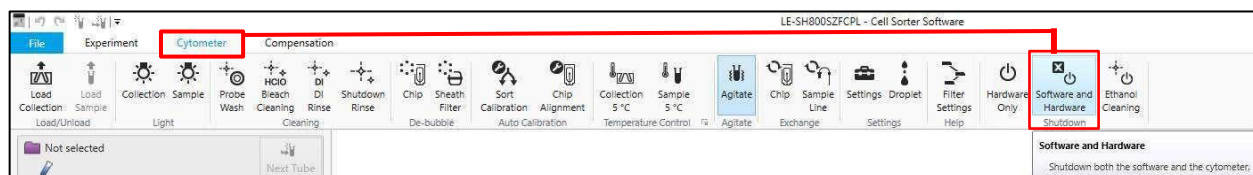
After autocalibration has been successfully completed, click *OK* to go to the *Create Experiment* window and proceed with your sorting experiment.

3. Sample Line Cleaning

Perform the following sample line cleaning after your sort is completed and if the instrument will **not** be shutdown. See Shutdown section below for a full shutdown of the instrument.

1 Perform Sample Line Rinse with Bleach and Water

- Go to *Cytometer* tab in the software.
- Choose *Software and Hardware Shutdown*.



- Follow the instructions for Sample Line Cleaning, making sure to choose "Normal Cleaning" for the bleach cleaning and the water rinse.
 - For the bleach cleaning, pour 10mL of 10% bleach (FACS Clean – yellow bottle) into a 15mL conical tube.
 - For the water cleaning, fill a 15mL conical tube with 12mL of H₂O from the PureLab system.
- Do not shut down the sorter, click "Cancel" when asked to perform shutdown.
- Go to file → Logout and be sure to keep the auto calibration results for the next user. Do **not** close the cell sorter software.

Note: The default nozzle size for the SH800 is 100 μ m. It is the responsibility of each user to ensure the 100 μ m is in place before the next appointment. If the 100 μ m nozzle is not in place, from the *Cytometer* tab, select *Chip Exchange* and follow on screen prompts.

4. Shutdown

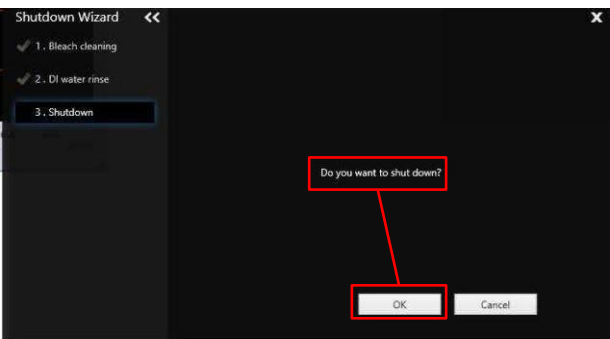
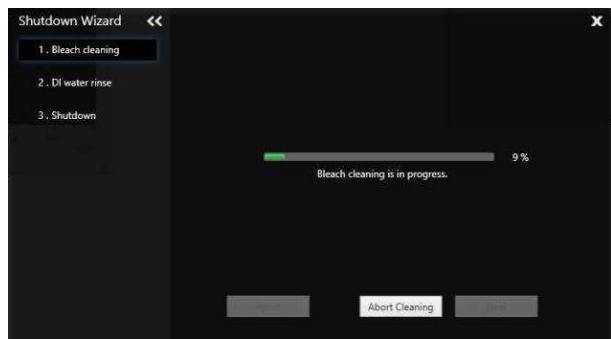
Perform shutdown if you are the last person of the day and:

- It is after 5 pm on weekdays or anytime on weekends and holidays.

1 Shut down the system.

- Go to *Cytometer* tab.
- Choose *Software and Hardware Shutdown*.
- Follow the steps for Sample Line Cleaning, making sure to check “Normal Cleaning” for the bleach cleaning and the water rinse.
 - For the bleach cleaning, pour 10 mL of 10% bleach (FACS Clean – yellow bottle) into a 15mL conical tube.
 - For the water cleaning, put in a 15 mL conical tube 12 mL of H2O from the PureLab system.
- Select *OK* when asked if you want to shut down the instrument.

The SH800 main unit and its software automatically shut down.



- When the instrument is fully shutdown, close the air valve as seen on page 1.

2 Wipe the inside of collection chamber, including the door and the deflection plates with 70% ethanol and kim wipes.

3 Turn off the hood fan, lights and outlet.

- To turn off the AMS, press the AMS blower button for a few seconds until it is off.

4 Clean the bench and workspace after using.

- Discard all the left over tubes and gloves in the proper bins before leaving. See Waste Disposal poster

| | Mon-Fri | Sat- Sun +Holidays |
|-----------|---|--|
| 12am-9-am | Perform Startup | Perform Startup If you are the first person of the day Perform Shutdown If you are the last person of the day |
| 9am | FCCF staff will perform the Startup | |
| 9 am-5pm | | |
| 5pm | FCCF staff will perform the Shutdown if no one is booked | |
| 5pm-12am | Perform Shutdown | |